The Value of Standards for Regulatory Product Approval Applications Incorporating Nonclinical and Clinical Gene Expression Microarray Data

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Challenges for integrating microarrays into drug development and medical practice*

- Scientific community
  - Demonstrating the strength of the linkage of genomic measurements to associated biological outcomes.
  - Demonstrating sufficient sensitivity, specificity, reproducibility, robustness, reliability, accuracy, precision, and clinical relevance of the chosen microarray platform application
- FDA
  - Developing early working relationships with stakeholders to provide reasonable and appropriate context-specific expectations

** Petricoin et al., Nature Genetics Suppl. 32: 474-9, 2002.*

To date, few sets of microarray data have been submitted to the FDA - concerns noted:

- Acknowledged variability in gene expression data generation, processing, and analysis between labs
- Acknowledged variability in data quality across platforms and between laboratories
- Acknowledged errors in probe sequence annotation
- Uncertainty over which data need to be submitted
- Uncertainties over how data should be submitted
- Uncertainties over how submitted data may be reprocessed by regulatory agencies
- Uncertainties over biological interpretation of data
- Uncertainty over regulatory action responses

How could Standards Help to Reduce Concerns Associated with Data Submitted for Regulatory Review?

- Acknowledged variability in gene expression data generation, processing, and analysis between labs
- *Tool to assess and reduce data variability between laboratories.*

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•Tool to assess and improve data quality

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- Uncertainty over which data need to be submitted
- Uncertainties over how data should be submitted
- *Providing data from performance standards may help to define and reduce data submission requirements*

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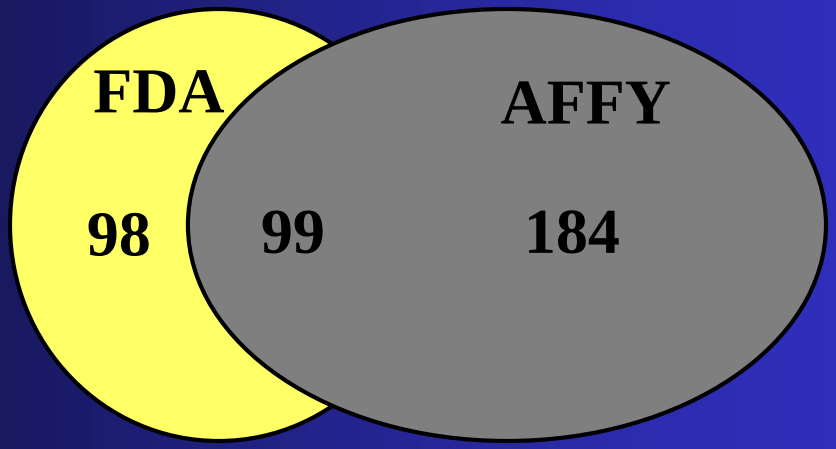
- Uncertainties over how submitted data may be reprocessed by regulatory agencies

- *Standards may provide agency statisticians with rationale for selection of most appropriate data analysis applications*

Points for Consideration

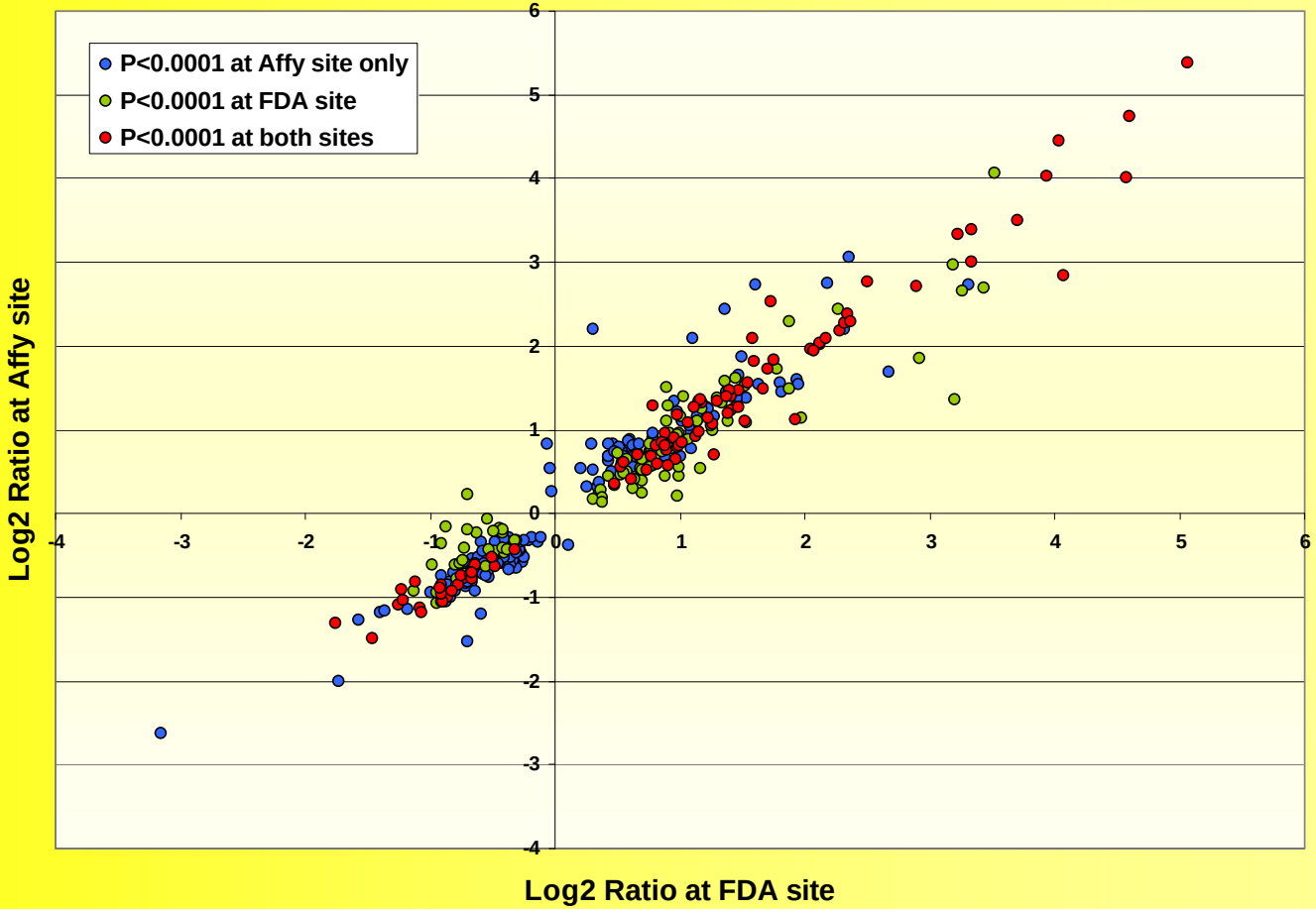
- *Microarray data submitted to the FDA will be generated from a variety of microarray platforms*
  - *Oligonucleotide and cDNA-based arrays*
  - *Commercial platforms*
  - *In-house custom arrays*
- *Cross-site comparisons (same RNA sample run on same platform in different labs) can often show discordance in number and type of significant gene changes.*

- ***Cross-Site Comparison: Same labelling, hyb, scanning, and data analysis protocols***



Sets of Significantly Changed Genes at P<0.0001
Control (n=6) vs Treated (n=5)

Scatterplot of Log2 Signal Ratios of Significant Genes with P<0.0001



r = 0.965 (union)

r = 0.985 (intersection)

- *Will it be necessary to standardize microarray protocols and data analysis methods in order to compare data across sites?*
- *How well can microarray data be compared against databases that were populated using dissimilar protocols?*
- *Given the complexity and variability of microarray data, can a standard be developed that would help assure the FDA that the platform and processor are capable of detecting the "biological truth" within a set of RNA sample comparisons?*

Standards to evaluate data quality

- Many metrics are routinely generated during microarray processing
  - *For RNA*
    - OD 260/280 ratio, 28s/18s ratio, 3'/5'ratios of control genes
  - *For Hybridization*
    - % Present Calls, Background levels, scatterplots for 2-color arrays

- What are the most informative metrics that can be submitted with microarray data to help reviewers assess data quality?

Standards to evaluate *platform (and laboratory) performance*

- Standard should be relatively invariant and regenerable.
- Some quality control checks built into platforms by manufacturers are specific to the array (e.g., probes for spike-in targets; non-mammalian genes for specificity) and therefore cannot be part of a universal standard. Standard should be formed on probes in common between platforms.
- Standard should resemble test samples (e.g., rat tissue standard for toxicogenomics) *and also* query a wide range of features on the arrays

- Universal RNA standards that are made from mixed tissues are used by many labs for intra-laboratory tracking of changes in sample processing, chip lot, scanner settings, or other lab performance issues over time.
- A set of mixed tissue standards that have varying ratios of components would contain real and quantifiable gene expression changes between samples and use endpoints measurable on most platforms

- *Examples of use of MTS*:
  - Samples containing marker gene transcripts that vary in concentration and type, e.g., Latin Square expts, are used to validate new versions of arrays or algorithms
  - MTS used to generate microarray standard data set for comparing data processing methods (He *et al*.)*

- What is the feasibility and the value of a mixed tissue standard for regulatory platform and data quality assessments?

* J. Bioinformatics, *in press*

FDA Office of Science & Health Coordination-Funded Collaborative Project:

Evaluation of Performance Standards and Statistical Software for Regulatory Toxicogenomic Studies

- *Performance Standards Laboratory Component (FDA)*
  - *K. Thompson, PI @ CDER*
  - *J. Fuscoe, PI @ NCTR*
- *Performance Standards Laboratory and Statistical Component (Outside Collaborators)*
  - *Rosetta Inpharmatics*
  - *Iconix*
  - *Affymetrix (Statistical support)*
- *Statistical Software Component*
  - *Education and training of FDA statisticians on statistical analysis of microarrays*

Proposed steps for testing feasibility of a performance standard using benchmark genes within mixed tissue samples

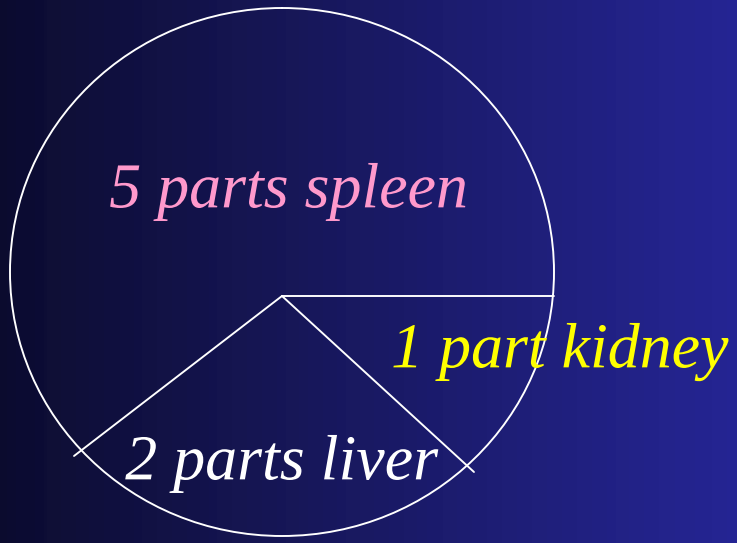
- Identify tissue-selective, low variance rat genes from control animal data in large databases (populated using a consistent protocol). These "benchmark" genes should optimally exhibit a consistent rank order of expression in defined samples (by tissue, age, sex, strain).
  - Analogous to compendium of gene expression in normal human tissues (HuGE Index). 19 tissue types on 121 GeneChip HuGeneFL arrays. Identified non-tissue selective maintenance genes and tissue-selective genes, ranked by expression level and variability.*
- Select tissues with large difference in number of tissue-selective genes from control animal data

**Hsiao et al., Physiol. Genomics 7: 97-104, 2001*

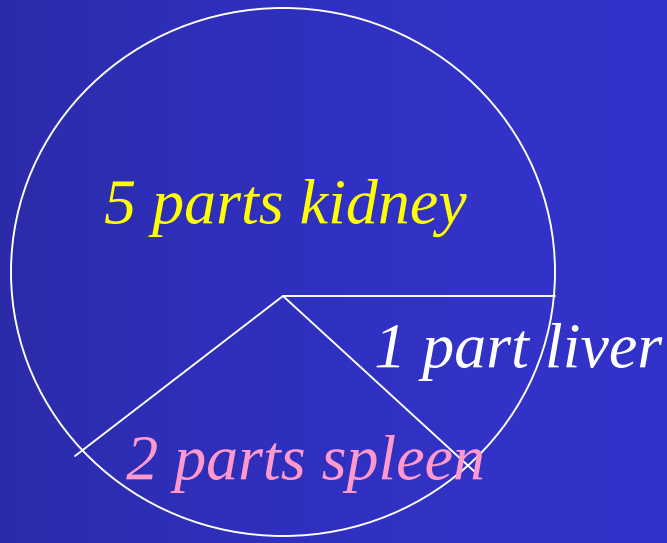
- Model a pilot set of tissue mixtures for the standard using database info and test on arrays
- Identify probe sets corresponding to benchmark genes on different platforms
  - Affymetrix GeneChips, in-house spotted 80-mer oligo arrays, Amersham CodeLink oligo arrays, Agilent custom 60-mer oligo arrays
- Determine consistency of rank order of benchmark gene expression across sites, samples, and platforms
- Evaluate the added value of exogenous spike-in standards (platform-dependent).

Hypothetical Mixed Tissue Sample Sets

Mixture 1



Mixture 2



Normalize on non-tissue-selective, invariant genes
Expected differences in tissue selective genes:
Spleen - 2.5-fold decrease,
Liver - 2-fold decrease, Kidney - 5-fold increase
May not correspond to platform performance capabilities

Expected Initial Outcomes

- Identification of probes that can perform similarly across platforms
- Determine normal range of false positive/false negative rates for MTS
- Determine normal range of lab-to-lab variance for MTS
- Determine normal range of cross-platform variance for MTS
- Publication of findings.

There is currently no regulatory requirement to generate microarray data on lead compounds. So, inclusion of standards with microarray data submitted to CDER is, at this point, voluntary, although when a scientific consensus is reached on the type, form, and value of a microarray standard for gene expression experiments, it may be recommended.

A cooperative framework among regulators, products sponsors, and technology experts is essential for realizing the revolutionary promise that microarrays hold for drug development, regulatory science, medical practice, and public health.*

** Petricoin et al., Nature Genetics Suppl. 32: 474-9, 2002.*